

FIGURE 1: Construct of Feline Thyrotropin beta-subunit with First Intron

1 30
 5'(GAA TTC)ATG ACT GCT ATC TAC CTG ATG TCC GTG CTT
 met thr ala ile tyr leu met ser val leu
 31 75
 TTT GGC CTG GCA TGT GGA CAA GCG ATG TCT TTT TGT TTT CCA ACT
 phe gly leu ala cys gly gln ala met ser phe cys phe pro thr
 76 120
 GAG TAT ATG ATG CAT GTC GAA AGG AAA GAG TGT GCT TAT TGC CTA
 glu cys met met his val glu arg lys glu cys ala tyr cys leu
 121 162
 ACC ATC AAC ACC ACC ATC TGT GCT GGA TAT TGT ATG ACA CGG
 thr ile asn thr thr ile cys ala gly tyr cys met thr arg
Intron 1
163 GTATGTAGTTCATCTCACTTCTTTAGCTGAAAATTAGATAAACCTAGACT
CAGTCCATTTCTATCCAGAAAGGAAATGAGATAAATCACAACCTCATTTACAG
ACCTAACGGTCATTGGCTCCTTAGAGGTAGAGTCCCTAGGTTATAATATACGGA
CCTACTCCATACAGTTGGTACAGATAATTTTACAATAGTTTACTCCCAAAGTT
TATTTAAACCTTATCTTGTTCCACGATCAAGGATAAAAGAGAGGTGTGTGTGT
ATGTCATTTTTTTTTGTCTCTATAGGATTCAGTGTGGATATGCTGAATTGGTATT
GGGGAATGGGACTAAGGAATCCTCCCCCAGTCCTATTTGTATCTATGGGATGT
AAGCGAATTAACATTTTGCTTCCTCTTCTGTGCTTCCCTCAG 580
 581 625
 GAT ATC AAT GGC AAA CTG TTT CTT CCC AAA TAT GCT CTG TCC CAA
 asp ile asn gly lys leu phe leu pro lys tyr ala leu ser gln
 626 670
 GAT GTT TGC ACC TAC AGA GAC TTC CTG TAC AAG ACT GTA GAA ATA
 asp val cys thr tyr arg asp phe leu tyr lys thr val glu ile
 671 715
 CCA GGA TGC CCA CAC CAT GTT ACT CCC TAT TTC TCC TAC CCG GTA
 pro gly cys pro his his val thr pro tyr phe ser tyr pro val
 716 760
 GCT GTA AGC TGT AAA TGT GGC AAG TGT AAT ACT GAC TAT AGC GAC
 ala val ser cys lys cys gly lys cys asn thr asp tyr ser asp
 761 805
 TGC ATA CAT GAG GCC ATC AAG ACA AAT GAT TGT ACC AAA CCC CAG
 cys ile his glu ala ile lys thr asn asp cys thr lys pro gln
 806 835
 AAG TCC GAT GTG GTA GGA GTT TCT ATC TAA (GCGGCCGC₍₄₎)(AT)₅-3'
 lys ser asp val val gly val ser ile stop

() denotes the Eco RI restriction sites

Bold denotes signal sequence***Bold/italic*** denotes the intron 1 sequence

FIGURE 2: Feline Thyrotropin alpha-subunit Construct(GAATTC) GCCCTT

1 45
AGT TAC TGA GAA ATC ACA AGA CGA AGC CAA AAT CCC TCT TCA GAT
 46 90
CCA CGG TCA ACT GCC CTG ATC ACA TCC TGC AAA AAG TCC GGA GGA
 91 135
AGG AGA GCC ATG GAT TAC TAC AGA AAA TAT GCA GCT GTC ATT CTG
 met asp tyr tyr arg lys tyr ala ala val ile leu
 136 180
GCC ATA CTC TCT GTG TTT CTG CAT ATT CTC CAT TCT TTT CCT GAT
 ala ile leu ser val phe leu his ile leu his ser phe pro asp
 181 225
GGA GAG TTT ACA ATG CAG GGG TGC CCA GAA TGC AAG CTA AAG GAA
 gly glu phe thr met gln gly cys pro glu cys lys leu lys glu
 226 270
AAC AAA TAC TTC TCC AAG TTG GGT GCC CCA ATT TAT CAA TGC ATG
 Asn lys tyr phe ser lys leu gly ala pro ile tyr gln cys met
 271 315
GGC TGC TGC TTC TCC AGA GCA TAC CCC ACT CCA GCA AGG TCC AAG
 gly cys cys phe ser arg ala tyr pro thr pro ala arg ser lys
 316 360
AAG ACA ATG TTG GTC CCA AAG AAC ATC ACC TCA GAA GCC ACA TGC
 lys thr met leu val pro lys asn ile thr ser glu ala thr cys
 361 405
TGT GTG GCC AAA GCC TTT ACC AAG GCC ACG GTA ATG GGA AAT GCC
 cys val ala lys ala phe thr lys ala thr val met gly asn ala
 Continued on next page

406 450
 AAA GTG GAG AAT CAC ACA GAG TGC CAC TGC AGC ACT TGC TAT CAC
 lys val glu asn his thr glu cys his cys ser thr cys tyr his
 451 459 492
 CAC AAG ATT (ATC GAA GGT CGT₍₁₎)(GAC TAC AAG GAC GAT GAC GAT
 his lys ile ile glu gly arg asp tyr lys asp asp asp asp
 493 495 510
 AAG₍₂₎ (TAA₍₃₎) (GCGGCCGC₍₄₎)(TATG)₅ 3'
 lys

Bold denotes 24 amino acid signal sequence as per structure in other species
Bold italics denotes sequence upstream from expressed but not secreted signal sequence that results in enhanced expression of the construct.

() denotes Eco R1 restriction site from TOPO Blunt vector

Underlined denotes additional sequence from TOPO Blunt vector

((1)) denotes Factor XA site

((2)) denotes Flag tag

((3)) denotes stop codon

((4)) denotes NotI restriction enzyme site

()₅ denotes extra bases needed for restriction enzyme to work

Figure 2 cont.

FIGURE 3: Yoked Feline Thyrotropin

1 30
 5'(GAA TTC)ATG ACT GCT ATC TAC CTG ATG TCC GTG CTT
 met thr ala ile tyr leu met ser val leu
 31 75
 TTT GGC CTG GCA TGT GGA CAA GCG ATG TCT TTT TGT TTT CCA ACT
 phe gly leu ala cys gly gln ala met ser phe cys phe pro thr
 76 120
 GAG TAT ATG ATG CAT GTC GAA AGG AAA GAG TGT GCT TAT TGC CTA
 glu cys met met his val glu arg lys glu cys ala tyr cys leu
 121 162
 ACC ATC AAC ACC ACC ATC TGT GCT GGA TAT TGT ATG ACA CGG
 thr ile asn thr thr ile cys ala gly tyr cys met thr arg
Intron 1
 163 GTATGTAGTTCATCTCACTTCTTTTAGCTGAAAATTAGATAAACCTAGACT
 CAGTCCATTTCTATCCAGAAAGGAAATGAGATAAATCACAACCTCATTTCACAG
 ACCTAACGGTCATTGGCTCCTTAGAGGTAGAGTCCCTAGGTTATAATATACGGA
 CCTACTCCATACAGTTGGTACAGATAATTTTACAATAGTTTTACTCCCAAAGTT
 TATTTAAACCTTATCTTGTTCCCACGATCAAGGATAAAAGAGAGGTGTGTGTGT
 ATGTCATTTTTTTTTGTCTCTATAGGATTCAGTGTGGATATGCTGAATTGGTATT
 GGGGAATGGGACTAAGGAATCCTCCCCCAGTCCTATTTGTATCTATGGGATGT
 AAGCGAATTAACATTTTGCTTCCTCTTCTGTGCTTCCCTCAG 580
 581 625
 GAT ATC AAT GGC AAA CTG TTT CTT CCC AAA TAT GCT CTG TCC CAA
 asp ile asn gly lys leu phe leu pro lys tyr ala leu ser gln
 626 670
 GAT GTT TGC ACC TAC AGA GAC TTC CTG TAC AAG ACT GTA GAA ATA
 asp val cys thr tyr arg asp phe leu tyr lys thr val glu ile
 671 715
 CCA GGA TGC CCA CAC CAT GTT ACT CCC TAT TTC TCC TAC CCG GTA
 pro gly cys pro his his val thr pro tyr phe ser tyr pro val
 716 760
 GCT GTA AGC TGT AAA TGT GGC AAG TGT AAT ACT GAC TAT AGC GAC
 ala val ser cys lys cys gly lys cys asn thr asp tyr ser asp
 761 805
 TGC ATA CAT GAG GCC ATC AAG ACA AAT GAT TGT ACC AAA CCC CAG
 cys ile his glu ala ile lys thr asn asp cys thr lys pro gln
 806 beta-specific primer sequence * CTP linker 850
 AAG TCC GAT GTG GTA GGA GTT TCT ATC CAG GAC TCC TCT TCC TCA
 lys ser asp val val gly val ser ile gln asp ser ser ser ser
 851 CTP linker 892
 AAG GCC CCT TCC GCC AGC CTT CCA AGC CCA ACG CGT CTC CCG
 lys ala pro ser ala ser leu pro ser pro thr arg leu pro

*reverse complement in construct

AFI III ligation
Site

893 CTP linker alpha-specific primer sequence 937 *
 GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA | TTT CCT GAT GGA GAG |
 gly pro ser asp thr pro ile ile pro gln phe pro asp gly glu
 938 977
 TTT ACA ATG CAG GGG TGC CCA GAA TGC AAG CTA AAG GAA
 phe thr met gln gly cys pro glu cys lys leu lys glu
 978 1022
 AAC AAA TAC TTC TCC AAG TTG GGT GCC CCA ATT TAT CAA TGC ATG
 Asn lys tyr phe ser lys leu gly ala pro ile tyr gln cys met
 1023 1067
 GGC TGC TGC TTC TCC AGA GCA TAC CCC ACT CCA GCA AGG TCC AAG
 gly cys cys phe ser arg ala tyr pro thr pro ala arg ser lys
 1068 1112
 AAG ACA ATG TTG GTC CCA AAG AAC ATC ACC TCA GAA GCC ACA TGC
 lys thr met leu val pro lys asn ile thr ser glu ala thr cys
 1113 1157
 TGT GTG GCC AAA GCC TTT ACC AAG GCC ACG GTA ATG GGA AAT GCC
 cys val ala lys ala phe thr lys ala thr val met gly asn ala
 1158 1202
 AAA GTG GAG AAT CAC ACA GAG TGC CAC TGC AGC ACT TGC TAT CAC
 lys val glu asn his thr glu cys his cys ser thr cys tyr his
 1203 1211
 CAC AAG ATT (ATC GAA GGT CGT₍₁₎)(GAC TAC AAG GAC GAT GAC GAT
 his lys ile ile glu gly arg asp tyr lys asp asp asp asp
 1245 1247 1262
 AAG₍₂₎ (TAA₍₃₎) (GCGGCCGC₍₄₎)(TATG)₅ 3'
 lys

* as written

Figure 3 cont.

KEY

() denotes the Eco RI restriction sites

Bold denotes signal sequence

Bold italics denotes intron 1 sequence 1=Factor XA site

(₁) denotes Factor XA site

(₂) denotes Flag tag

(₃) denotes stop codon

(₄) denotes NotI restriction enzyme site

(₅) denotes extra bases needed for restriction enzyme to work

Figure 3 cont.

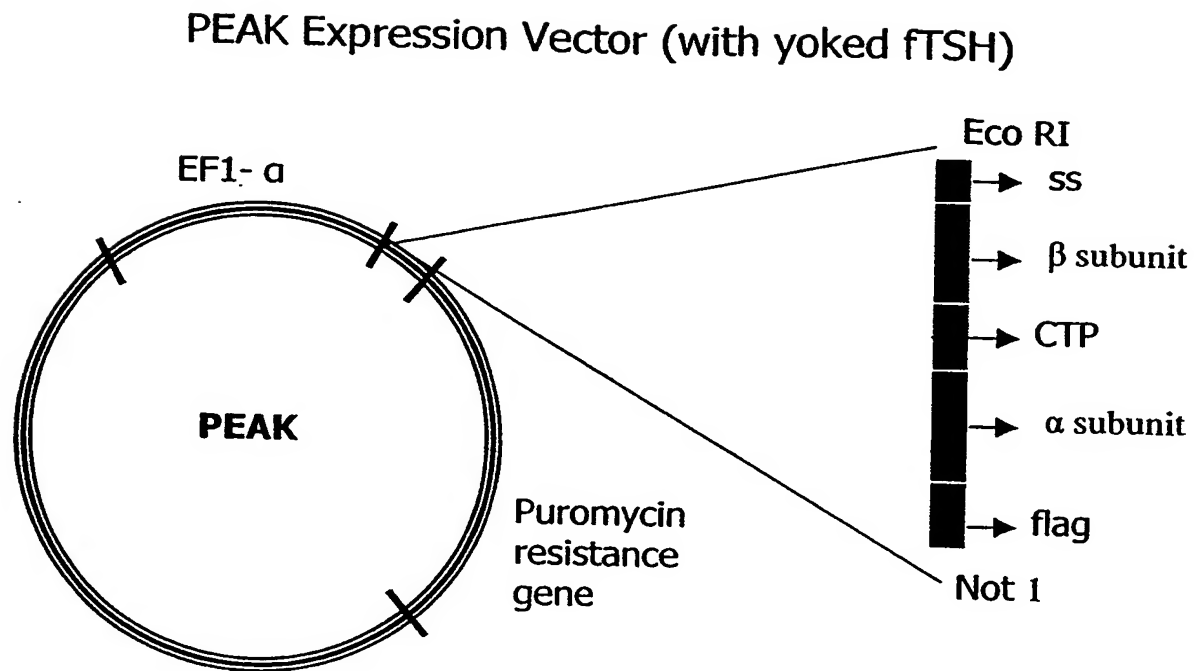


Figure 4



Figure 5a

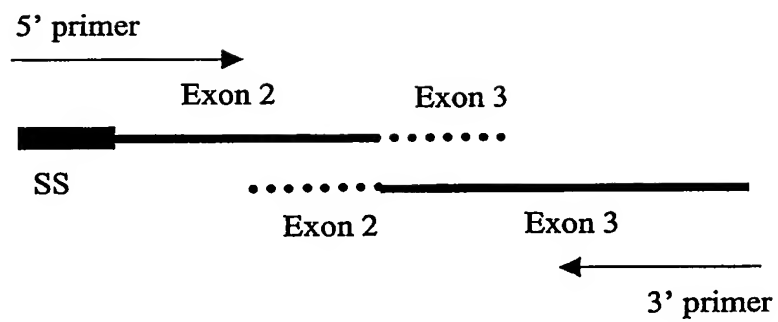


Figure 5b

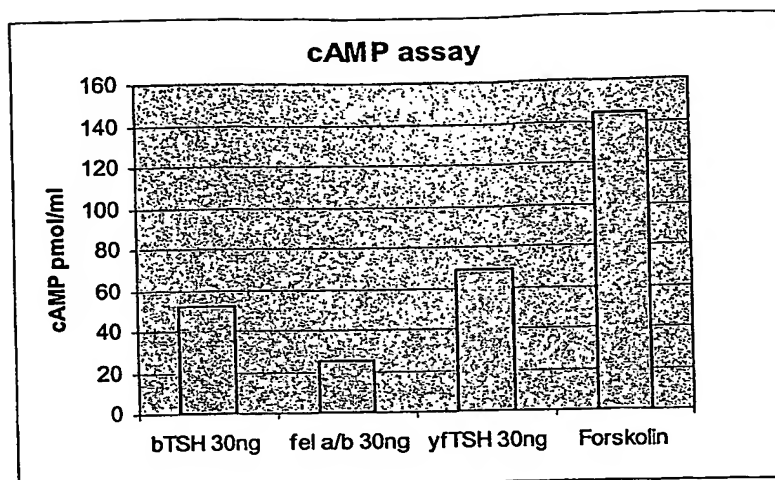


Figure 6a

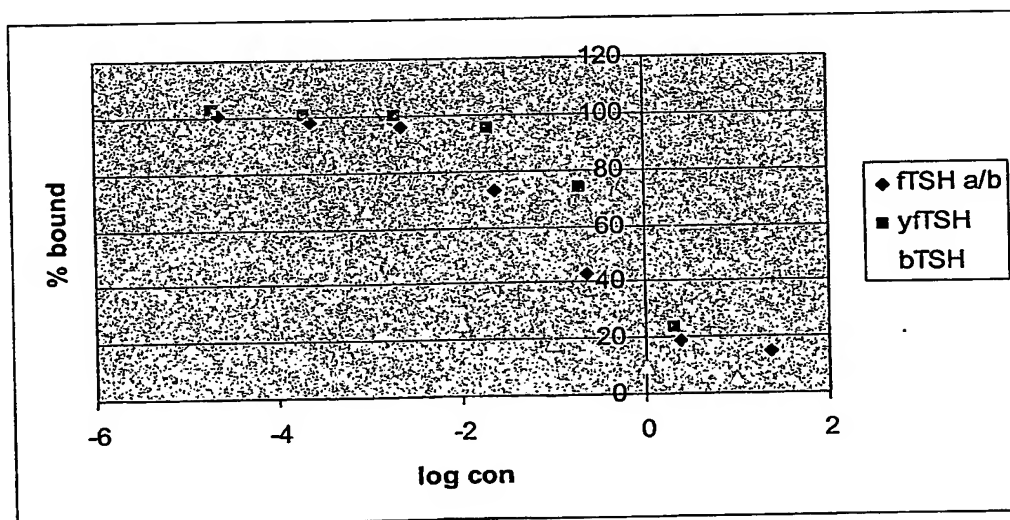


Figure 6b